ON THE ORIGIN AND DEVELOPMENTAL POTENTIALITIES OF BLOOD CELLS*

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THE nature and variety of the functions performed by the cells of the blood, in health and in disease, make the problems concerning their origin, development, interrelationship, and ultimate disposition of fundamental interest to medical investigators and of vital importance to the clinician. The blood elements are influenced by, and in turn affect every normal physiologic as well as every abnormal pathologic reaction. For this reason the recording of the blood cells has long since become a universal routine in clinical medicine; but this very fact has tended frequently to minimize the value and limit the interpretations which can and should be made from such data—familiarity, and careless or inexpert technique tending perhaps to breed a certain contempt for the diagnostic and prognostic value of this information. G. Lovell Gulland,[1] however, in his 1930 Harveian Oration on the Circulating Fluid, prophesied "the time when the differential count will be more important than the auscultation of the heart." For some, at least, this time has already arrived.

While Neumann first described in 1868 hemocytogenesis in the bone marrow, significant interest and effective progress in hematology dates only from 1891, when Ehrlich reported the differential tinctorial reaction of the cells of the blood to specific dyes. There followed a quarter century of intensive effort to establish working hypotheses from the new morphologic criteria thus made available, which resulted in the emergence of two broad, general, divergent and controversial interpretations: the monophyletic and the polyphyletic doctrines, with many individual modifications (Fig. 1). Embryologic studies only contributed to the confusion, and there gradually arose an intricate and extensive descriptive nomenclature, based upon minutiae of morphologic detail relating to dead cells, from which the average medical student and practitioner has not yet recovered. With the introduction, however, of the methods and

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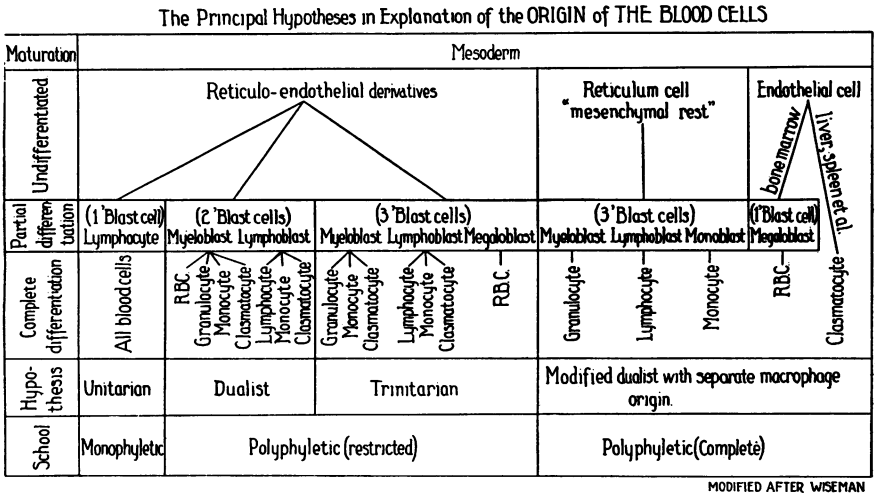

Fig. 1

tools of modern experimental physiology, including the cultivation and study of living explanted tissue *in vitro* by Harrison (1913), the development of the technique for observing hemocytogenesis in the living chick blastoderm by Sabin[2] (1920), the application of certain non-toxic vital and supravital dyes to the study of living cells by Goldmann (1909-12), Evans and Schulemann (1914-15), Simpson (1922) and Sabin (1923), the *in vitro* determination of the respiratory quotients of cells under varying conditions by Warburg (1930), the controlled nutritional studies of Whipple in dogs (1920), and the inauguration of basic clinical investigations of the human anemic and leukemic states—with the perfection of these procedures, hematologists began to accumulate a fund of dynamic data, which quite naturally has supplemented, modified and clarified the more limited morphologic speculations of the past.

In this particular domain of medicine, it is especially important to temper interpretations based upon morphology with pertinent functional observations. Throughout the mammalian life span, the cells of blood and connective tissues arise as primitive, undifferentiated, mesenchymal elements requiring a period of complex maturation, with almost infinite potential, and actual morphologic variation before the definitive units become functionally available to the body. These maturative phenomena have proved to be fertile soil for the multiplication of exceedingly involved hematopoietic hypotheses, far more complicated in morphologic

fancy than the more recently accumulated physiologic facts would seem to justify. I shall, therefore, devote little or no time either to attacking or defending any system of terminology, or in discussing the purely morphologic basis for cell classification. Rather, I shall invite your attention to certain broad, general principles of blood cell growth and blood cell responses, an understanding of which should permit each physician to envisage for himself the underlying hematopoietic and tissue reactions in disease, on the basis of currently obtainable data from the newer types of blood cell *studies* (not "counts").

Five fundamental questions, peculiar to and inherent in hematopoietic tissue, have formed the focus for all of the more recent physiologic studies of the phenomena involving the blood cells: (1) the nature and significance of cell origins; (2) the functional specificity and interrelationships of each definitive cell type; (3) the factors essential for differentiation and cell maturation; (4) the forces governing cell delivery and distribution; (5) the conditions influencing cell destruction.

Embryologic Hematopoiesis

Embryologists are in general agreement that the blood cells derive originally from the mesodermal rather than the ectodermal or entodermal cell layers (Fig. 2). It is also now accepted that the first blood cells to form in the embryo are the red cells, and that they arise intravascularly. Danchakoff[3] and Maximow[4] both affirmed this in 1908 and 1909 using fixed tissues. Sabin[2] in 1920 actually observed in continuous studies of the living chick blastoderm of the second day of incubation, mesoderm differentiating into angioblasts, which in turn gave origin to the first hemoglobin synthesizing cells, the earliest endothelium, and the first blood plasma; megaloblasts, thereafter, arose from and developed within the vascular endothelium of the area vasculosa.

Knoll[5] (1927-29) in a series of thirty-nine human embryos, obtained also in the living state and studied for the development of the hematopoietic tissues, found that the blood contained only red cells through the second month of gestation. Granulocytes appeared about two and one-half months after the red cells, and the lymphocytes still later. Only by the third month were white cells to be seen in appreciable numbers in these human embryos. During the third month the formation of the blood cells is taken over by the liver. By the latter part of the third and during

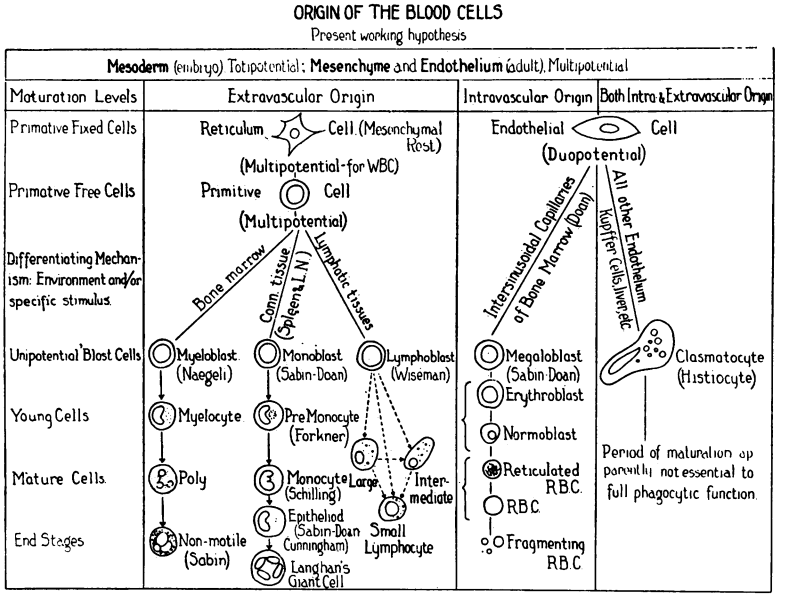

Fig. 2

the fourth month changes begin in the cartilage cells in the center of the shaft of the long bones. Vessels from the perichondrium penetrate into the zone of softening cartilage, carrying along connective tissue elements. The primitive mesenchymal cells of the marrow at this stage were accurately described and beautifully illustrated by Maximow[6] in 1910.

Bone Marrow as an Organ

In the hypoplastic areas of adult human marrow, this same simple organization of the marrow parenchyma is maintained and when analyzed, the tissue may be seen to be composed only of fat cells, vascular endothelium, and reticulum, i.e. mesenchymal, cells. Such a marrow provides the starting point, as Peabody[7] reëmphasized in 1926, for approaching an understanding of cell origins and cell relationships in the more densely hyperplastic areas of active red marrow. According to one school of hematologic thought, as the need for increased red blood cells arises, the fat cells in a quiescent sector are rapidly demobilized, the endothelium lining the intersinusoidal capillaries hypertrophies and divides, with the intravascular appearance of megaloblasts; subsequently these basophilic cells with vesicular nuclei and large single nucleoli, multiply as

they gradually elaborate hemoglobin and eventually form the typical scattered, circumscribed intravascular islands of erythroblasts and normoblasts so characteristic of "red" marrow. Full maturity embodies loss of pyknotic nucleus coincident with development of complete complement of hemoglobin and cell delivery through recanalization of the non-patent erythrogenic capillary. If the need is for granulocytes and thrombocytes, the mesenchymal "rests" (reticulum cells) show increased mitoses with the appearance of myeloblasts and megakaryocytes forming the center of extravascularly located foci of myeloid multiplication and maturation.

The Lymphocyte Question

Another interpretation of such hematopoietic activity attributes to one, common, free, stem cell, a so-called lymphocyte, multi- or totipotentialities for all other definitive blood elements. The principal division of opinion at the present time would seem to hinge upon the conception, or the definition, of a "lymphocyte." Probably no amount of discussion, based solely upon morphologic detail, can ever effectively settle the points at issue. There are none of the more distinctive characteristics, such as hemoglobin, specific granules, or patterned vacuoles to differentiate sharply the so-called small circulating lymphocyte of normal blood from other simple, undifferentiated mononuclear elements. Moreover, certain it is that each definitive strain of blood cells originates from a simple, relatively less differentiated, basophilic mononuclear cell, and only gradually evolves or elaborates its distinguishing cytoplasmic and nuclear characteristics. The farther back in the life cycle of any blood or connective tissue cell the hematologic investigator goes, the harder it is to differentiate on morphological grounds alone. Indirect, as well as direct, means must often be utilized in ascertaining essential identifying data. Pappenheim's original concept of a common stem cell, the lymphoidocyte, for all the blood cells, while receiving frequent reaffirmation (Maximow,[4] Danchakoff,[3] Weidenreich, Jordan,[8] Ferrata, Latta and Ehlers, Bloom, Lewis and Lewis) has also been frequently challenged (Ehrlich, Naegeli, Schridde, Schilling, Krumbhaar, Sabin, Cunningham and Doan, Peabody, Clark and Clark, Seeman, Tischtschenko, Hall and Furth). Jordan and his associates in a series of studies (1920-1936) of hematopoiesis in the frog, conclude that the differentiation of lymphocytes originating from reticulum cells gives rise to all of the various types of definitive blood cells. Tischtschenko[9], on the other hand, working in

His' laboratory in Berlin (1931) concludes that the myeloid and lymphoid elements in frog's blood "must belong unconditionally to two different hemocytogenic systems." Bloom, at the University of Chicago, has cultured the cells from thoracic duct lymph and observed the transformation of large and small lymphocytes into inflammatory polyblasts[10] (1928) and granulocytes[11] (1937). The Clarks[12] (1930) at the University of Pennsylvania, and Seemann[13] (1930) working under Aschoff's direction at the Pathologic Institute of the University of Freiburg, have been unable to interpret in this way their observations; and Seemann, after an extensive cytologic survey in the rat, reports that "the real lymphocyte from lymphatic tissues is not capable of transformation into monocytes and histiocytes," and states, furthermore, that the "extreme monophyletic school of Maximow places under the category of lymphocytes entirely different forms of cells, which are *only separated by supravital staining or biological experiment.*" This was the conclusion of Sabin, Cunningham and Doan[14] earlier (1922-1925) working at Johns Hopkins with the supravital technique and *in vivo* animal experimental procedures. These investigators defined and differentiated the life cycle of each of the definitive strains of blood cells, ascribing to the specific endothelium lining the intersinusoidal capillaries in marrow the intravascular origin and development of the megaloblast and its progeny; to the extravascular mesenchymal reticulum, the "primitive free cell," lymphocyte-like in size and superficial characteristics, from which the myeloblast, lymphoblast and monoblast derive under appropriate conditions. An extended and comprehensive series of experiments followed at Harvard and at the Rockefeller Institute, correlating cell form with cell function under both physiologic and selected pathological conditions, which included the significant studies of Wiseman[15], all tending to establish the specificity of the life cycle and the functional independence of the so-called small lymphocyte of blood and lymph nodes. Hall and Furth[16] (1938) using thoracic duct lymph from normal dogs, and from normal, tuberculous, and B. monocytogenes-infected rabbits, found no evidence in extensive tissue culture studies of any transformation of lymphocytes into monocytes or fibroblasts. Occasional monocytes (0.1 to 2.3 per cent) as well as small (85 to 90 per cent), intermediate, and large lymphocytes (5 to 13 per cent) were encountered in the thoracic duct lymph collected for culture. In our own laboratory for the past several years, Dr. Houghton has been culturing the lymphocytes obtained from pa-

tients with lymphatic leukemia, leuko-lymphosarcoma, and infectious mononucleosis, among materials from other sources, and there has been no evidence adduced in any single instance which could be interpreted as representing a true differentiation of the type of cell recognized morphologically, metabolically and culturally as the "lymphocyte." Rather has the evidence continued to accrue in support of the concept of the functional importance of the lymphocyte *per se* in the body economy as a definitive unit, which frequently serves to antagonize, or reciprocate with, rather than evolve into other cell types.

It would seem, therefore, that the various points of view which have been expressed about the lymphocyte might best be amalgamated and integrated through the acceptance of at least two components as comprising the "lymphocyte" of the monophyletic school: the one, the classical small lymphocyte of blood, lymph nodes and spleen which rarely if ever changes its fundamental form; and the other a comparably small lymphocyte-like, 'blast cell which may always be found associated with active white cell differentiation and maturation anywhere.

Appropriating the universally accepted morphologic criteria of cell maturation common to the better known hemocytogenic cycles, viz., decreasing cytoplasmic basophilia, changes in number, form and distribution of mitochondria, disappearance of nucleoli with progressive elaboration of nuclear chromatin and cell motility, Wiseman[17] divided arbitrarily the circulating blood lymphocytes into three morphologic groups; designated them Young, Mature, and Old; and established their mean frequency and relative proportionate occurrence under conditions of health in animal and human subjects. In normal human adults quantitative values for lymphocytes were observed to range from 750 to 3,000 per c.mm., the *qualitative* formula in the average healthy individual being far more constant: Y : M : O : : 4 : 48 : 48. Thus, although the number of circulating lymphocytes fluctuates widely in health from person to person and in the same individual from time to time, the proportionate age relationships, based upon the morphologic characteristics cited, remain much more constant and have seemed to change significantly only when disease intervenes. From the evidence available, Wiseman has assumed that the absolute number of lymphocytes present in the peripheral blood at any one moment is the resultant of the balance between four major forces: (1) the speed and efficiency of differentiation and maturation of lymphocytes within the follicles of the lymph

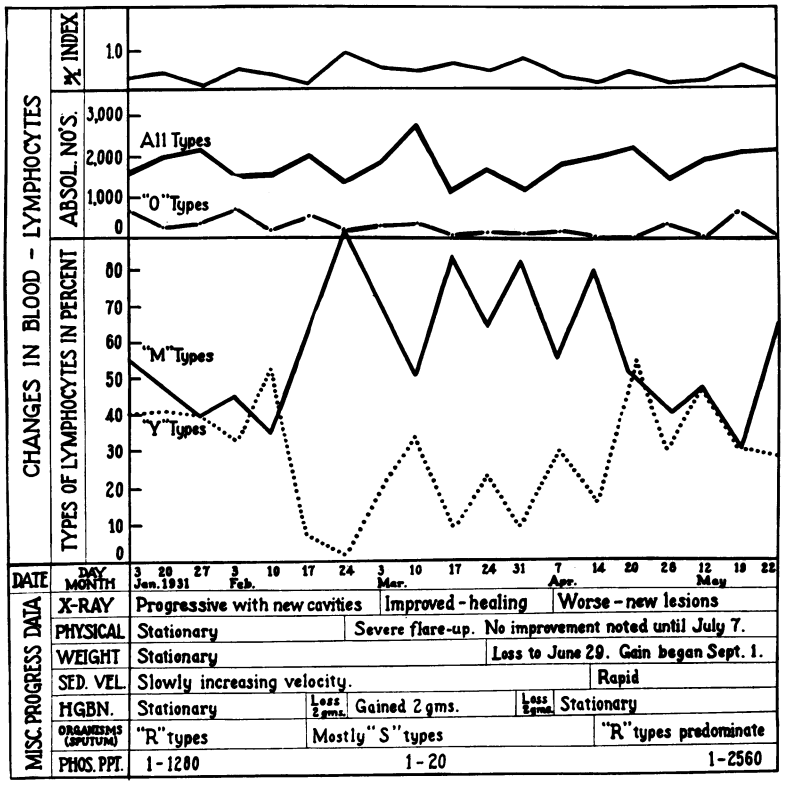

Fig. 3

W. I., white male, aged 22 years, with known clinical tuberculosis of 3 years duration, and a sanatorium history of 11 months. X-ray diagnosis: far advanced active tuberculosis with multiple cavitation. The clinical and laboratory findings are recorded. The Y:M:O qualitative relationships within the lymphocyte strain of cells changed markedly during the 5 months of observation, reflecting more sensitively than any other single criterion, the patient's reaction to his disease.

nodes; (2) the rate of delivery of these cells to the circulation; (3) the rapidity of their withdrawal or destruction, reflecting changing functional demands or toxic influence; (4) the capacity for reservoir storage of lymphocytes in the tissues. It is entirely conceivable, and in practice it has been found to occur, that a profound imbalance in the lymphopoietic equilibrium may occur without alterations in the total circulating lymphocytes, beyond the rather wide limits established for normal individuals, quite as has been recognized for the neutrophilic granulocytes since the analyses of Arneth, Schilling, and a host of subsequent observers. This relative age-maturity interpretation of the finer differentiating qualitative criteria observed in fixed and supravitally stained films of

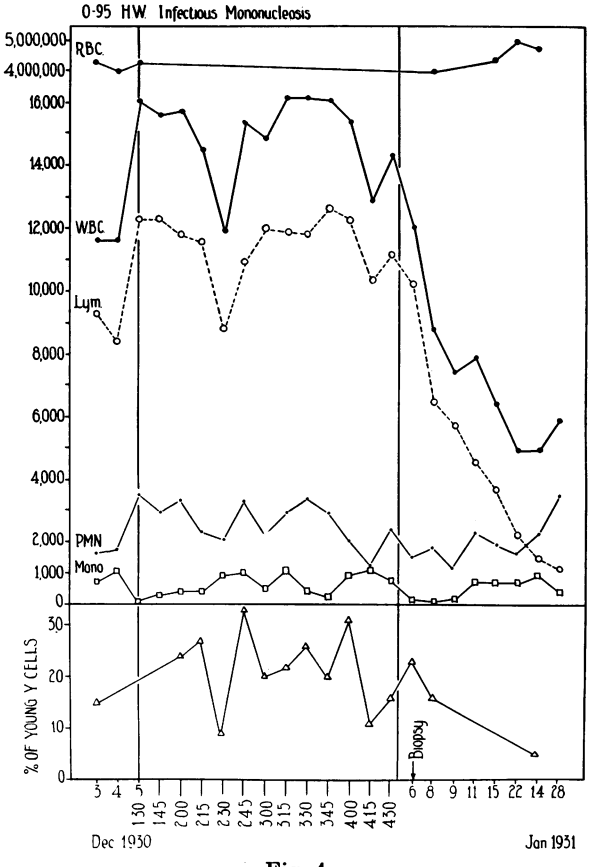

Fig. 4

The young, relatively large, lymphocyte with Reider nucleus, which usually dominates the blood picture in infectious mononucleosis, is pathognomonic of this benign dyscrasia. Serial, 15 minute interval counts were made during the period indicated on Dec. 5 to demonstrate the physiologic correlation (nonleukemic) between the fluctuations in the total lymphocyte count and the corresponding proportion of "young" lymphocytes. As the disease subsided and the lymphocytes returned to normal in number and quality, the neutrophils, which had been moderately depressed, returned to their normal number and relationship in the differential count.

normal blood could, of course, only be established and then applied clinically by submitting the hypothesis to experimental and practical tests under known conditions. This Wiseman has proceeded to do. In rabbits injected with foreign proteins or inoculated with bovine tubercle bacilli the profound disturbances in lymphocytes—reciprocal to granulocytes in the former instance and to monocytes in the latter—were only fully understood and could only be adequately correlated with the final tissue studies by recognizing and interpreting the qualita-

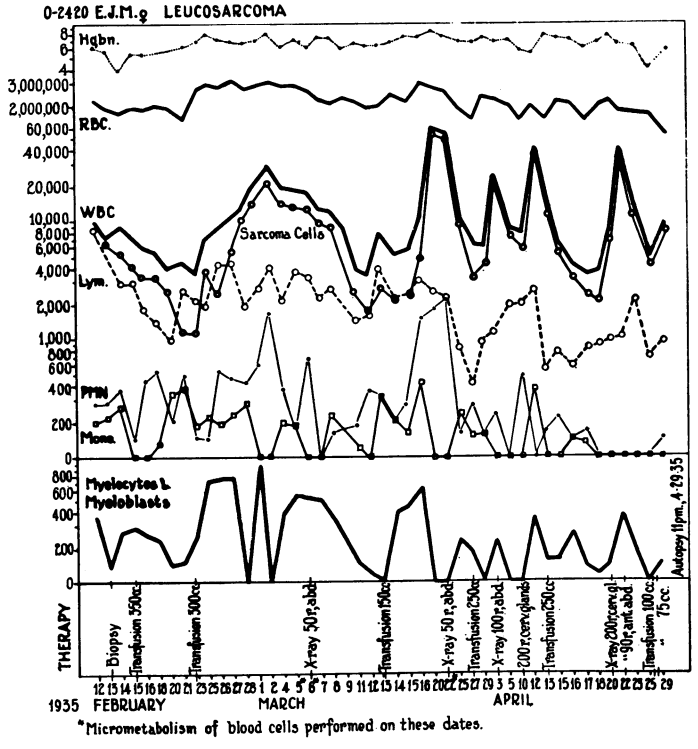

Fig. 5

In leukosarcoma, two distinct types of lymphocytes may be seen side by side in the same microscopic field; the one, normal mature, according to every criterion; the other, obviously a much younger form, with large single nucleolus and characteristic cytoplasmic changes when observed with supravital stains. Deep x-ray therapy in very small dosage destroys promptly the "sarcoma" lymphocytes, while depressing little, if any, the normal lymphocytes. A myelophthisic blood picture, with anemia, nucleated red cells, myeloblasts and myelocytes, reflects the hyperplasia of lymphosarcoma cells in the bone marrow. Micrometabolic studies have shown a malignant type of respiration for the "sarcoma" cells, in sharp contrast to the data obtained from normal and leukemic lymphocytes.

tive, in addition to any quantitative changes in the lymphocytes. In human tuberculous patients (Fig. 3),[18] in infectious mononucleosis, (Fig. 4), in leuko-lymphosarcoma (Fig. 5), in chronic lymphatic leukemia (Fig. 9),[19] the importance, significance and validity of the qualitative differentiation of the lymphocytes has been clearly established. The interpretations based upon these morphologic criteria in the circulating lymphocytes have been validated by and correlated with basal and cell metabolic studies, specific cell motility characteristics, by observed variations in cell behavior in tissue culture, and by contrasting biopsy and postmortem tissue. That is to say, the human blood lymphocyte shows a

variety of characteristic cellular alterations more or less pathognomonic of various disease mechanisms, but always it retains sufficient criteria to identify it with its own definitive maturation cycle.

The Granulocytes

Turning now to the granulocyte, a much more readily recognized maturation cycle may be seen in normal bone marrow. The agranular myeloblast has a less deeply basophilic cytoplasm than the germinal center lymphoblast, fewer and finer mitochondria, and a nucleus usually with more nucleoli. Seldom need the trained cytologist hesitate in differentiating lymphoblast and myeloblast. The more primitive free cell common to lymph node, bone marrow, and connective tissues[14] and only observed under conditions of excessive stimulation in one or other of these areas, is the cell, if any, which will be confused with the small lymphocyte of suspected multipotential capacities of differentiation. As soon as the first specific granules appear in the myeloblast—neutrophilic, eosinophilic or basophilic—the respective definitive cell type may be predicted. Coincident with granule appearance, but not before, neutrophilic and eosinophilic myelocytes become oxidase reactive, the amount of the peroxidase precipitate being directly proportional quantitatively to the number and size of the granules present in any given cell[20]; basophil granules remain oxidase negative throughout the basophil granulocyte life cycle. Lymphocytes throughout their life cycle and in their various pathologic responses, and all "primitive" and 'blast blood cells remain oxidase negative. All myelocytes lack intrinsic motility until maturity under normal circumstances, which is to say, from the morphological standpoint, until a full complement of specific granules has been developed, the basophilia and mitochondria of the cytoplasm have gradually diminished to the vanishing point and the nucleoli have disappeared and the chromatin greatly condensed in a nucleus which is beginning to constrict preparatory to lobulation. In acute infections and under certain other pathologic conditions, motile, granule-deficient neutrophilic leukocytes with mitochondria and basophilic cytoplasm, may be encountered. Throughout the myelocytic phase of maturation, the cytoplasmic criteria are the more dependable, especially granule development; less than ten specific granules per cell being designated myelocyte A, approximately 50 per cent granules, myelocyte B, and a full complement of granules, myelocyte C.[21] During this inverse reciprocal evolution in

mitochondria, cytoplasmic basophilia and specific granule formation, the nucleus becomes smaller, more pyknotic and finally segments. Ameba-like motility ensues with a liquefaction of the hitherto gel-like cytoplasm, and the relative age of the motile mature granulocyte is thereafter gauged by the number of lobes to the nucleus (Arneth[22]). The origin of the myelocytes being extravascular in the parenchyma of the bone marrow in the normal human adult, and the function of the mature granulocytes being accomplished in remote tissues or along the mucous surfaces of the body, intrinsic cellular motility is a prerequisite to the entrance of the leukocytes into, and their egress from the blood stream, except on occasions secondary to pathologic marrow hyperplasia.

The Monocyte-Clasmatocyte Relationship

The monocyte or "transitional" of Ehrlich has been almost as difficult a cell to accept and to assign specificity and independence as the lymphocyte. First, its azurophilic granules in a mottled basophilic cytoplasm with oval or slightly indented vesicular nucleus were thought to represent an earlier stage in the maturation of the myelocyte, later the azur granules of the lymphocytes were thought to be identical with them.[23] In 1913 Schilling[24] first declared that the criteria were sufficient to establish the monocyte as an independent and distinct cell entity, and the development and application of the supravital staining technique to this problem of cytologic specificity subsequently has fully supported the rightness of Schilling's conclusions. A cytoplasmic system of vacuoles was revealed in the living cell, which stains characteristically with neutral red, is arranged as a rosette opposite the *hof* of the nucleus, and is surrounded by mitochondria, all of which methyl alcohol obliterates from the ordinary fixed stained blood films. Furthermore, a characteristic surface film type of motility differentiates the monocyte in fresh preparations from both granulocyte (ameboid) and lymphocyte (peristaltic). Here again, a careful study of the peripheral nodes[25] and spleen,[26] where monocytes arise normally, and more especially the analysis of experimental monocytoses[27] establishes a maturation sequence from primitive, basophilic, non-vacuolar, non-motile, monoblast through succeeding stages of nuclear and cytoplasmic development to characteristic mature, motile monocytes. Under pathological conditions, such as tuberculosis, the monocyte may be altered by the bacterial lipids[28] to become the typical epithelioid cell and Langhan's giant cell of the tissue tubercle.

Now, morphologically and functionally distinct from the other circulating leukocytes by common consent, the monocyte still must share the connective tissue role of phagocytosis and thereby merge its identity, in the opinion of some, with the tissue macrophage or clasmatocyte. Whether these functionally and histologically similar, but not morphologically identical, cells represent different phases in the life cycle of the same cell, or whether there are two tissue phagocytes of different origin remains today a matter of debate and further experimentation. The evidence from supravital studies of experimental and pathological tissues favors the independent origin and separate identity of monocyte and clasmatocyte (Fig. 2), though recognizing at times their common response and similar behavior in non-specific tissue reactions. The clasmatocyte is the one cell of the tissues, and/or rarely of the blood, which does not seem to require a period of maturation for full functional activity.

So long as abstract static morphologic studies alone were made of the complex medley of multiple cell types, each with its own complicated life cycle, little progress of functional or clinical significance was possible; only unverifiable speculation existed. However, with the acquisition of increasingly distinctive criteria of type and age specificity, and with the means and the interest to analyze and interpret a variety of induced and spontaneously occurring cellular *disequilibria* in terms of functional responsiveness and relative tissue adequacy, substantial physiologic information of great significance has become available, and facts are slowly beginning to fortify or undermine fancies, as the case may be. With the newer knowledge, of fundamental mechanisms and inhibiting and stimulating influences upon blood cell multiplication and maturation, the formulation and application of rational prophylactic, as well as therapeutic regimes, has become possible.

Erythropoiesis

In no area of specific or general health is this more true than that of the oxygen-carrying mammalian red blood cell. Other than a relative degree of regional anoxemia, we do not yet know the additional fundamental requisites for the primary initiation of erythrogenic differentiation. When we do, the answer to some of the "primary" aplasias will probably be known. Many toxic substances will inhibit or destroy normal erythropoiesis, such as benzol, arsphenamin, x-ray, and radium;

and when promptly recognized and eliminated, recovery ensues. A congenital accentuation of physiologic splenic hemolysis may disturb the hemolytopoietic equilibrium to the point of acute hemoclastic crisis; the bone marrow is hyperplastic, usually at the late erythroblast and normoblast level of maturation, and successful splenectomy initiates a prompt erythrocyte reëquilibration, with complete clinical recovery.[29] In iron deficiency states, a hypochromic, microcytic anemia, with low plasma iron,[30] without intrinsic marrow defect may be remedied specifically through adequate replenishment of the body's depleted iron reserves. In such an instance no diminution in number of circulating or marrow red cells need exist, but iron must be available for the synthesis of hemoglobin. Vitamin C and thyroxin are also essential to the maintenance of normal erythropoietic equilibrium. When maturation arrest occurs at the megaloblastic level of red cell differentiation, an hyperplasia develops in the marrow with progressive peripheral anemia, hyperchromic and macrocytic in type. The life cycle of the erythrocyte is best observed while following the recovery from such a state (Fig. 6). Because of inability to utilize iron without the erythrocyte maturation factor, plasma or transport iron values are higher than normal.[30] Coincident with the supplying of this deficit by any one of a number of oral or parenteral sources of the active principle, iron once more is utilized in considerable quantities, reflected by a precipitous fall in the plasma iron level in direct relationship to the maturation, and, therefore, prompt disappearance of the megaloblasts as such from the marrow; in their place appears a transitory increase in early erythroblasts to be followed promptly by late erythroblasts with still more iron containing hemoglobin until, finally, by the fifth or sixth day, normoblasts dominate numerically the age-range of the nucleated red cells in the marrow with only a minimal number of the less mature erythroid elements still present. In the peripheral blood the reticulocytes usually reach their peak some 24 to 48 hours after the normoblasts have become the predominating red cell in the marrow, representing the youngest of the enucleated red cells in the circulation, and marking the culmination of the suddenly reëstablished maturation cycle. As erythrocytic equilibrium is again approached, the reticulocytes fall to their physiologic level of less than 1 per cent, and the marrow gradually resumes its normal cellular relationships, permitting the myelophthisically depressed platelets and granulocytes to regain their accustomed levels. In the patient

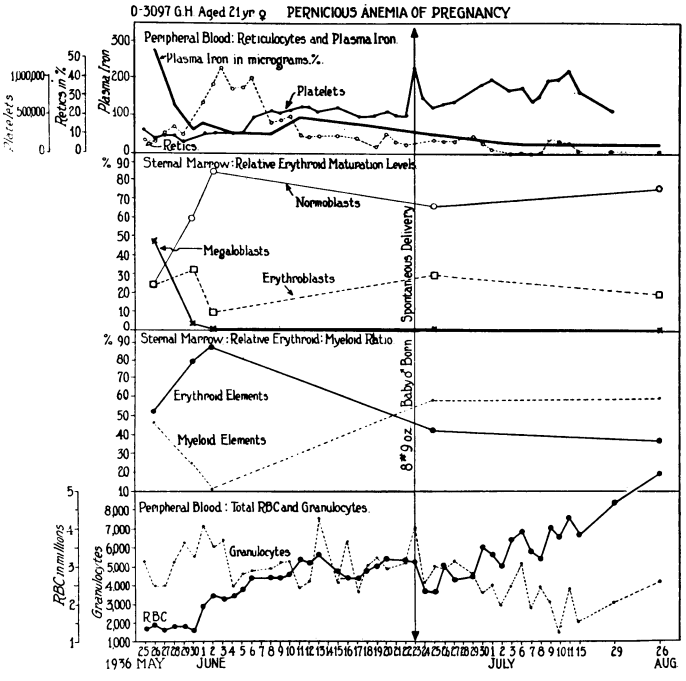

Fig. 6

"Extrinsic factor," dietary deficiency during the fifth successive pregnancy in as many years, resulted in a macrocytic anemia in this patient indistinguishable, as to peripheral blood picture, hyperplastic megaloblastic marrow and high plasma iron, from Addisonian pernicious anemia. Gastric analysis revealed 68° free HCL. The institution of a high caloric, protein diet plus 50 gms. autolyzed yeast daily brought about a prompt utilization of the excessive circulating plasma iron; this was reflected immediately in the bone marrow by increased hemoglobin synthesis, which reduced the proportion of megaloblasts; the resulting erythroblasts in turn gave way to normoblasts, tc be followed by the usual reticulocyte peak in the peripheral blood. With the reduction in marrow erythroid hyperplasia, platelets and granulocytes returned to their normal levels, and, with the reestablishment of a normal red cell and hemoglobin equilibrium, plasma iron and reticulocytes became normal, and the erythroid : myeloid ratio in the marrow became physiological. An identical maturation sequence has been observed in repeated instances of adequately treated primary pernicious anemia with "intrinsic factor" deficit.

illustrated in Fig. 6, the approximate length of time for maturation from megaloblast to non-reticulated mature red blood cell might be estimated at 15 days, on the basis of the available data. Jones[31] has described the megaloblast and its interrupted maturation in Addisonian pernicious anemia as a distinctly abnormal pathologic phenomenon, bearing no relationship to, and having no counterpart in, embryologic or normal adult erythropoiesis. This has not been the interpretation of Dr. Sabin and her associates, who rather consider the macrocytic deficiency anemias to represent the result of a metabolic deficit compar-

able to the simple iron deficiency states, in which no inherent bone marrow pathology as such exists. The functional inadequacy of marrow is secondary to gastrointestinal or liver pathology; the last-named assumes primary etiologic significance in the light of Castle's, Whipple's and Minot's classical observations. Given suitable erythrocyte maturation factor, an entirely normal bone marrow, histologically and functionally, results promptly.[32]

Hemocytologic Reciprocities

Although many of the same noxious agents which depress erythropoiesis may inhibit or destroy granulocytes and lymphocytes and interfere with thrombocytopoiesis, it has become increasingly apparent that highly specific effects, either stimulatory or inhibitory, may be limited solely or largely to one cell strain. There is also evidence that significant reciprocal cell-strain relationships frequently result as a part of pathologic reactions.[33] The simplest and most readily understood example of marrow cell reciprocity is that just cited of the mechanical limitation of myelopoiesis and thrombocytopoiesis in pernicious anemia in relapse secondary to megaloblastic hyperplasia. The same diminution in normal number of circulating blood elements, with characteristic "left shift" qualitative alterations in the cells, is seen in multiple myeloma, metastatic carcinoma, osteosclerosis, leukosarcoma, the leukemias, or in any condition where such an abnormal appropriation of marrow parenchyma restricts the origin and development of normal hemopoietic foci. The remedy for specific blood cell insufficiencies resulting from these causes, lies obviously in restricting the abnormal cell growth and not in the institution of hemopoietic therapeusis *per se*.

However, more subtle reciprocal relationships among the blood cells have been observed which have a direct bearing upon the question of cell origins and their developmental potentialities and specificities. From the experimental angle it has been found that the amphophilic (neutrophilic) granulocytes in rabbits may be strongly stimulated by the nucleic acid derivatives and their components, the nucleotides, specifically, adenine and guanine.[34] A general myelocytic marrow hyperplasia resulted, and under prolonged stimulation ectopic foci of myelocytes were found in spleen and kidneys.[35] It was noted during these studies that the total number of circulating lymphocytes decreased as the granulocytes rose, and at postmortem the lymph nodes, spleen and

other sites of lymphoid development were found to be practically devoid of all follicles and all germinal center activity.[33] No myelopoietic activity and no myelocytic infiltration had occurred in these areas, so that the atrophy appeared to be the result solely of spontaneous, reciprocal lymphocytic hypoplasia. Conversely, the administration of foreign protein to rabbits[17] resulted in a marked increase in circulating lymphocytes at the expense of granulocytes, the latter falling as low as 400 per c. mm. during the peak of lymphocytosis. Autopsy surveys showed an extensive, marked, generalized hyperplasia of all lymphoid tissues including a greatly enlarged spleen, while the bone marrow everywhere showed a marked myelocytic hypoplasia extending to complete aplasia in some normally active areas.

Simple, uncomplicated, hypertherm- or radiotherm-induced fever in rabbits has been found to influence myelopoiesis favorably while at the same time destroying lymphocytes and inhibiting their regeneration temporarily.[36] A progressive "left shift" in the nuclear index of the circulating granulocytes was correlated with prompt progressive, myeloid hyperplasia and increased mitotic activity in marrow, and a marked immediate postfebrile leukocytosis; conversely the peripheral lymphopenia reflected a progressive cellular destruction necessitating increased clasmatocytic phagocytosis without regenerative replacement wherever lymphocytes were located in the tissues, with a prolonged latent postfebrile period before lymphopoiesis could again become effectively reëstablished.

In each of the instances cited, one particular cell strain was markedly responsive to a stimulus, which at the same time, either directly or indirectly, influenced adversely another supposedly closely related cell type. It might seem that were the granulocytic cells directly dependent upon the lymphocyte of blood and lymph nodes for their origin and differentiation, any influence subversive to lymphocytic integrity would likewise be reflected in a diminished production and supply of granulocytes to the organism. Or, if an unusually prolific supply of multipotential lymphocytes became available, there would presumably be no obvious reason for a reciprocal decrease in the direct progeny of such a stem cell; rather some corresponding increase in granulocytes might be anticipated. Quite the opposite has been observed in the experimental field.

Is there, then, any counterpart in the cellular disequilibria occur-

ring during the clinical course of human disease, which might permit of interpretations in one direction or the other concerning fundamental cell origins and cell relationships? In infectious mononucleosis a marked lymphocytosis (Fig. 4), both relative and absolute, usually occurs with characteristic qualitative changes in this cell strain, associated with a more or less generalized lymphadenopathy. A reciprocal granulopenia has been observed, resulting not infrequently in a profound decrease to 500 or less granulocytes per c. mm., the sternal bone marrow showing at such times an absolute decrease in myelocytes.[33] Clinical recovery is not complete until the disturbed cell relationships in the blood and tissues have been corrected.

The Mechanism and Specificity of Cell Stimulation and Cell Inhibition

Turning to the problem of acute granulopenia in human disease, the nucleotides may be said to have the strongest and most powerful chemotactic and maturative stimulus for neutrophilic granulocytes of any agent thus far studied. In patients with the Schultz syndrome,[37] if monocytes remain present in the peripheral blood and the sternal bone marrow is not entirely aplastic for myeloid elements, recovery more often than not parallels the administration of pentnucleotides. This statement is made with full realization of the establishment of a specific hypersensitive destructive and inhibitory etiology for amidopyrine and certain other drugs in certain specific myelopenic states, with the desirability and necessity of prevention in these cases. In all leukopenic patients, in which the etiology is not immediately obvious, however, a knowledge of the state of the bone marrow is essential to intelligent therapy today. Usually this information may be obtained quite as satisfactorily by sternal puncture as by surgical trephine. The principal requirement is a familiarity with the morphologic characteristics of the maturation cycle of each strain of cells found normally in bone marrow and with the relative proportions of each as revealed by actual differential cell counts of representative marrow samples.

When the peripheral leukopenia is found to be secondary to both a maturation arrest and an inhibition of marrow myelopoiesis, the recovery, if it occurs, is reflected by changes in the granulocytes, which are entirely comparable to those observed in the red cells during the recovery from relapse in pernicious anemia. Depending upon the degree

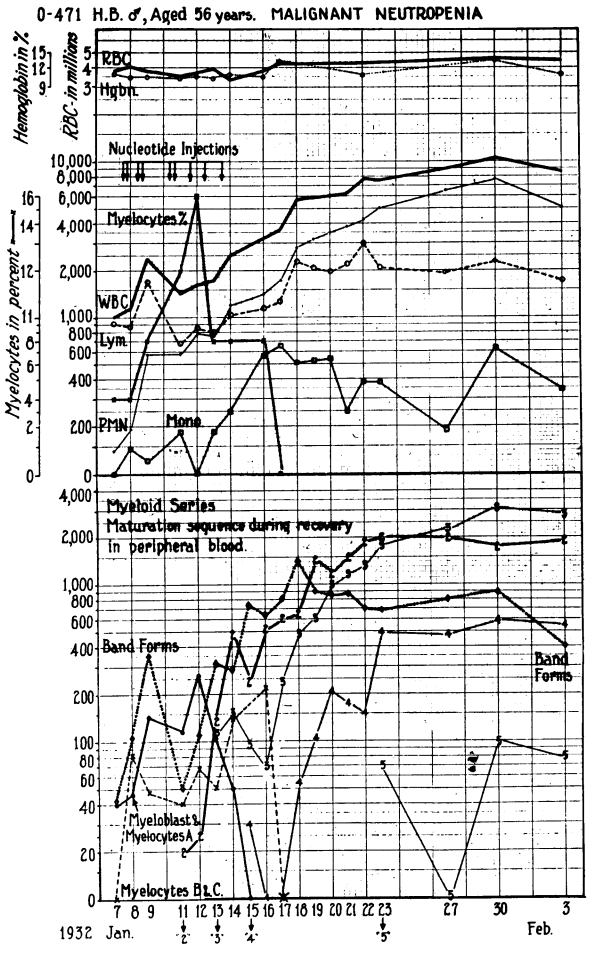

Fig. 7

This, in detail, is the peripheral blood reflection of bone marrow recovery in a classical acute neutropenic episode of undetermined etiology. There appeared sequentially in the blood every stage in the entire life cycle of the neutrophilic granulocyte, from myeloblast to mature, five-lobed polymorphonuclear, the myelocyte percentage rising to a peak and then dropping sharply in duplication of the reticulocyte phenomenon illustrated in Fig. 6. From such observations it is estimated that it may take as long as 16 days for maturation from myeloblast to five-lobed neutrophil, and that regeneration must take place, in some instances at least, from the myeloblast level.

of myelocytic immaturity which prevails at the time of the reactivation of the marrow, there will appear transitorily in the peripheral blood, myelocytes in increasing percentage, comparable to a reticulocyte peak.[38] Moreover, the maturation sequence from myeloblast through myelocytes A, B, and C may be followed both in serial marrow punctures and in the daily white cell differential counts. In one such patient

(Fig. 7), when first seen in an acute granulopenic episode with less than 1000 leukocytes per c. mm. (erythrocytes and thrombocytes normal), myeloblasts and myelocytes A were present in the peripheral blood. During the days following the institution of nucleotide therapy, there appeared a maturation sequence in the circulating myeloid elements, which reflected accurately the steps of recovery in the marrow. The myeloblasts and the earliest myelocytes with the first few specific granules increased to their highest point of 280 per c. mm. on the fifth day of treatment, disappearing on the eighth. Myelocytes B and C, containing a more complete complement of specific granules, were present at the end of the first 24 hours, reached their maximum of 250 on the ninth day, and had disappeared by the following day. The "band forms" of Schilling, the youngest of the motile mature granulocytes, were present to the number of 40 per c. mm. on the first examination and were found in all preparations at all times, but did not show any sustained absolute increase until the eighth day, reached their peak of 1400 on the eleventh day, and thereafter gradually decreased to resume their proportionate representation as equilibrium was again established. The first granulocyte with a segmented nucleus, two lobes, was observed on the fourth day, and thereafter these two-lobed neutrophils increased steadily until the twentieth day, when the three-lobed granulocytes, which had been present since the sixth day, finally surpassed them in total number. One cell with four lobes to the nucleus was seen in one preparation on the eighth day, but not until the eleventh day were they permanently present. Five days later, on the sixteenth day following the beginning of recovery, granulocytes showing five nuclear segments appeared, at which time the total granulocytes had increased from their original low of less than 100 to something over 5000 per c. mm. Thus, from myeloblast to five-lobed polymorphonuclear neutrophilic leukocyte required approximately 2 weeks' time in this individual. With increasing maturity of the circulating myeloid cells came increased absolute numbers, reflecting both a specific myelopoietic inhibition and maturation arrest in the marrow during the fastigium of clinical symptoms. The episode described, occurred in January, 1932 unrelated to any determinable drug etiology, and though two less severe leukopenic relapses occurred during the following two years, associated with severe emotional environmental stresses and strains, this physician-patient is still living and well at the time of this writing, with no evidence of hemo-

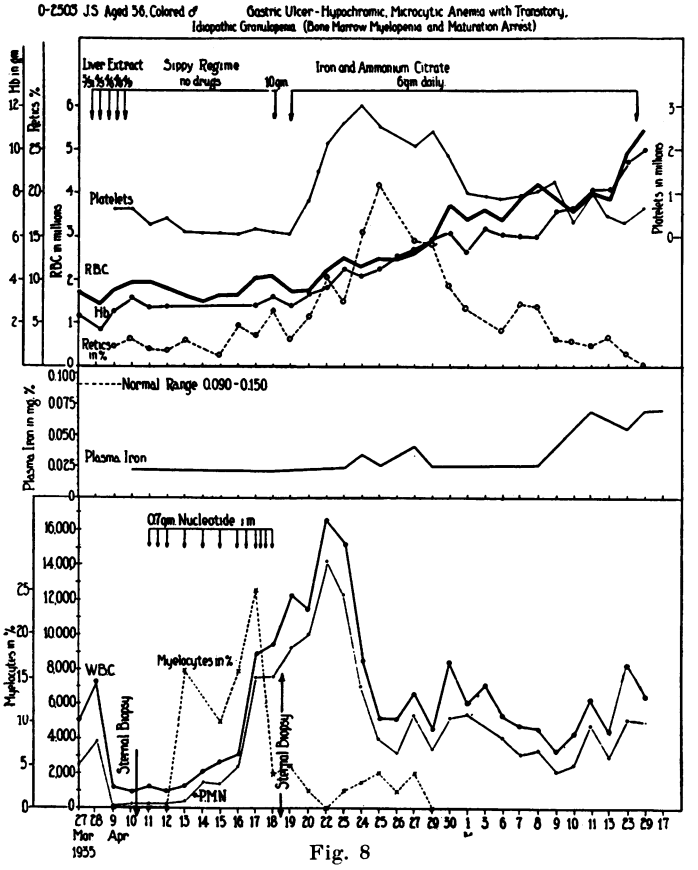

Fig. 8

Demonstration of the complete independence of myelopoietic and erythropoietic responses to specific therapy in an individual developing an acute idiopathic granulopenia while in the hospital under observation for a severe anemia secondary to bleeding peptic ulcer. Liver extract neither helped the anemia nor prevented nor "cured" the neutropenia. The extremely low plasma iron reflects the exhaustion of the body's iron reserves from chronic hemorrhage resulting in a typical, microcytic, hypochromic anemia. Note different time relationships in the administration of the various therapeutic agents and the corresponding cellular responses, including the platelet increase which accompanied the erythroid recovery. See Table I for the actual differential counts of blood and sternal marrow cells April 10 and 18.

poietic marrow inadequacy.

The specificity of the inhibition of marrow myelopoiesis in these clinical instances of granulopenia has been stressed, thus emphasizing two significant points with reference to the physiology of bone marrow as an organ, namely, the probable independence of the respective stem cells from which erythrocytes and granulocytes normally arise throughout postembryonic life, and, an obvious corollary, the specificity of the responses to both depressants and stimuli of these respective cell strains. Fig. 8 and Table I further support these conclusions. During

TABLE I

(To be studied in conjunction with Fig. 8 and the text)

NUCLEOTIDE-TREATED IDIOPATHIC NEUTROPENIA
(maturation arrest)

Serial Sternal Bone Marrow Biopsies

Patient: J. S. Aged 36 years, colored, ♂.

Diagnosis: 1—Gastric ulcer—hypochromic microcytic anemia.
2—Idiopathic granulopenia, acute, transitory (no drugs).

April 10	1935	April 18

BLOOD

Total WBC	925		9400	
Total RBC.....	1,700,000		2,090,000	
Hemoglobin			2.8 gm./100 cc.	
(Newcomer) ..	2.5 gm./100 cc.	PMN	81%	Lym. small 8%
Supravital Diff..	Only 1 2-lobed PMN found	PMB	1	int. 1
		Myelocytes	3	Monocytes 6

BONE MARROW

Supravital Diff.	First Biopsy			Second Biopsy		
PMN	13	4%		76	14%	
Metamyelocyte	6	1%	15%	68	12%	94%
Neutrophilic "C".....	30	9%		360	67%	
"B".....	246	71%		32	6%	
"A".....	51	14%	85%	0	0%	6%
Basophilic "C".....	1			12		
"B".....	13			4		
"A".....	10			2		
Eosinophilic "C".....	1			8		
"B".....	1			2		
"A".....	0			0		
Myeloblast	1			0		
Lymphocyte	2			0		
Monocyte	1			2		
Clasmatocyte	16			4		
Primitive Cell	0			0		
Total WBC	392		36%	572		54%
Normoblast	595	87%		354	75%	
Erythroblast	62	9%		116	24%	
Megaloblast	23	3%		4	0.8%	
Total RBC	680		64%	474		46%
Cells counted	1072			1046		

8.4 gm. nucleotide given i.m. in 8 day period

hospitalization, incident to a severe hypochromic, microcytic anemia secondary to bleeding peptic ulcer, there developed in this patient an acute granulopenic episode. Sternal biopsy proved the accuracy with which the peripheral blood was reflecting the underlying marrow pathology. Only 36 per cent of all nucleated marrow elements were of the myeloid series and 85 per cent of these were the very young myelocytes A and B, a distinct "left shift" from the normal maturative relationships. The nucleated red cells (64 per cent of all marrow elements) were predominantly (87 per cent) at the normoblastic level, which is representative of the maturation level when no inadequacy in the erythrocyte maturation factor prevails, and explains the lack of response to liver extract, given as a control. Eight days later, when the granulocytes had increased in the peripheral blood from practically zero to 8000 per c. mm., a second sternal biopsy from another interspace revealed a corresponding change in the age and proportionate representation of the myeloid elements without significant qualitative alteration in erythropoiesis or circulating erythrocyte level. The myelocytes represented 54 per cent of all nucleated cells at this time, and of equal or even greater significance was the increase in the relative proportion of the more mature myelocytes C and metamyelocytes from 15 per cent to 94 per cent. During this change in the number and maturity of myelocytes in the marrow, the peripheral blood was showing a myelocyte peak with a high of 25 per cent reached on the seventh day following institution of nucleotide therapy, preceding the absolute increase in total circulating granulocytes, much as does the reticulocyte rise following specific therapy in the anemias. During this episode, erythrocytes and hemoglobin remained stationary. However, with the institution of adequate iron therapy in this patient, a typical reticulocyte response was elicited, reaching a high of 21 per cent on the seventh day followed by a rapid return to normal in plasma iron, total red cells and hemoglobin. No further hematologic disturbances have been observed in this patient.

The occasional chronic lymphatic leukemia patient will have a bone marrow hypoplastic for myelocytes unassociated with lymphocytic hyperplasia or infiltration. In such a case[39] it was possible to stimulate a rise in neutrophilic granulocytes from a few hundred to 10,000 per c. mm. with daily injections of the nucleotides (Fig. 9). No demonstrable effect on the lymphocytes was observed, suggesting the essen-

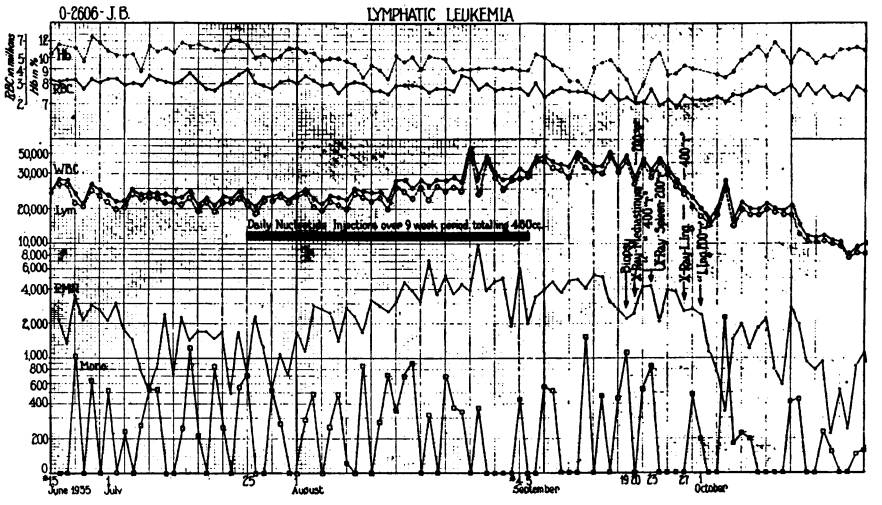

Fig. 9

A patient with chronic lymphatic leukemia with reciprocal granulopenia as low as 500 neutrophils per c.mm., during 9 weeks of daily nucleotide injections showed a gradual but steady increase in these elements to 10,000 per c.mm. During this period there was no reciprocal fall in the leukemic lymphocytes, but rather a slow uninterrupted increase, which continued until deep x-ray therapy was administered. The polymorphonuclear neutrophils failed to continue their upward trend as soon as nucleotide therapy was discontinued, and subsequently fell to their previous low level, indicating the specific character of the stimuli, and independent capacity to respond, of these two cell strains.

tial independence of lymphocytic and myelocytic responses, at least under certain circumstances. The same fundamental observation has been made relative to lymph node atrophy and lymphocytic hypoplasia in chronic myeloid leukemia. In this disease a profound peripheral lymphopenia is the rule, which reflects the reciprocal cell relationships as found in the hemopoietic tissues. Again, in certain patients showing pan-marrow hypoplasia, with anemia, lymphocytic leukopenia, a mild lymphadenopathy, and the presence of a scattering of lymphocytes in the otherwise aplastic bone marrow, this spontaneous "pseudoleukemic" myeloid-lymphoid reciprocity has at times raised the question of specifically stimulated lymphatic hyperplasia.

Reports of an occasional toxic disturbance of normal marrow function during sulfanilamide medication are appearing in the medical literature. One such instance in our own experience serves to emphasize the difference in character and in time relationships of the various marrow element responses when such a reaction occurs. A 66 year old, colored, male patient entered the hospital, on the fifth day following mild trauma, critically ill with a proven beta-hemolytic streptococcus

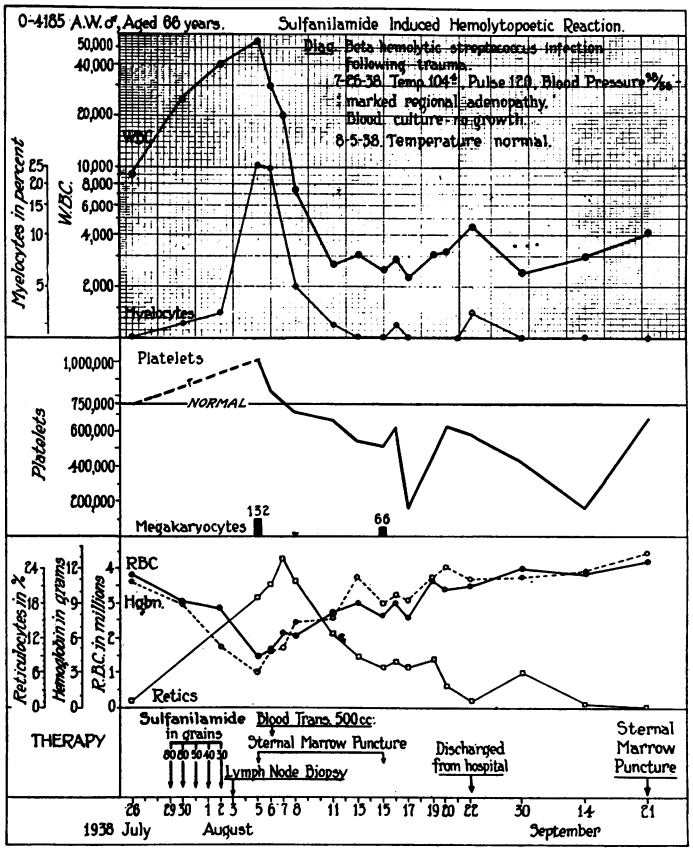

Fig. 10

This patient illustrates the difference in type, degree and time relationships of the reaction of the various bone marrow elements to the same toxic agent. At the point of maximum hemolysis, with red cells at a million and a half, hemoglobin 3 gms., and a spontaneous reticulocyte peak of 24 per cent, the total white cell count was 55,000 with 27 per cent myelocytes, and the platelets were over a million per c.mm. By the time the red cells had again reached a normal equilibrium a persistent leukopenia and thrombocytopenia had developed. See Table 2 for the correlation of peripheral blood and sternal bone marrow data taken at representative periods.

infection involving the affected extremity and the regional lymph nodes. The total white cells were only 9000 per c. mm., granulocytes 85 per cent with 11 per cent band forms and numerous toxic granule cells; red cells and reticulocytes were within normal limits. Sulfanilamide was administered in diminishing dosage as indicated in Fig. 10 for 5 days, during which period clinical improvement was prompt and striking, and coincided with a marked rise in circulating white blood cells to a high point of 55,000, with 65 per cent neutrophilic granulocytes, 25 per cent

TABLE II

(To be studied in relation to Fig. 10 and the text)

SULFANILAMIDE INDUCED HEMOLYTOPOIETIC REACTION

Serial Sternal Bone Marrow Biopsies

Patient: A. W. Aged 66 years, colored, ♂.

Diagnosis: Beta hemolytic streptococcus infection following trauma. Marked regional adenopathy. Blood culture—no growth.

1938	August 5	August 15	September 21

BLOOD

Total WBC.	55,000	2,500	4,250
Total RBC.	1,570,000	2,660,000	4,250,000
Total Platelets	1,070,000	526,680	680,000
Hemoglobin	3 gm./100 cc.	9 gm./100 cc.	13.2 gm./100 cc.
Reticulocytes	19.8	7.4	0.2
Supravital Diff.			
PMN	65%	60%	58%
PMB	0	—	1
Myelocytes	27	0	0
Monocytes	0	10	8
Lymphocytes	8	30	33

BONE MARROW

Supravital Diff.	**First Biopsy**	**Second Biopsy**	**Third Biopsy**
PMN	73%	48%	46%
Neutrophilic "C"	22.5	18	36
"B"	2	2	0
"A"	0	2	0.5
PMB	0.5	2	0.5
PME	1	6	0.5
Eosinophilic "C"	0	0	0.5
"B"	0	0	0.5
Myeloblast	0	1	0
Lymphocyte	0	14	13.5
Monocyte	0	0	0.5
Clasmatocyte	**1**	**5**	**0.5**
Plasma Cell	0	2	1
WBC Ratio	**27%**	**29%**	**74%**
Normoblast	68%	50%	44%
Erythroblast	32	50	56
Megaloblast	0	0	0
RBC Ratio	**73%**	**71%**	**26%**

280 grains sulfanilamide given orally in 5 days (July 29-Aug. 3).

of which were myelocytes. The platelets were found at this time to be somewhat higher than normal. The red cells meantime had fallen sharply to a million and a half, the hemoglobin to 3 gms. per 100 cc., while the reticulocytes, reflecting an increased spontaneous compensatory marrow delivery of new young erythrocytes, had risen to 19.8 per cent. As the red cells rapidly increased on the rebound, the leukocytes fell to a definite leukopenic level, which was maintained for some 6 weeks thereafter. The platelets paralleled the falling white cells and remained below normal for a comparable period. On the first two sternal marrow studies, the myeloid cells represented less than 30 per cent of all nucleated cells encountered, while by September 21 the proportion of erythroid to myeloid elements had again returned to normal. Thus, there were stimulatory, inhibitory and destructive phases in the reactions, differing in degree and duration with the cell types involved. In this instance one may hypothesize the sulfanilamide as increasing the effectiveness of, and the need for phagocytic leukocytes, at the same time diminishing the toxic myeloid inhibiting effects of the streptococci; conversely, the drug proved hemolytic for red cells in this individual in the dosage given, with the development of an acute, transitory anemia. Following the subsidence of the infection and the elimination of the therapeutic drug, the marrow returned more or less promptly to its original equilibrium.

Much more information needs to be accumulated relative to the white cells before we shall be in a position to influence their life cycles at the source as beneficially as is now possible for the red blood cells. At the moment, the important fact is their apparent independent response to various physiologic and pathologic influences, and their separate, even though closely related, functional specificities and capacities.

The Hemolytopoietic Equilibrium

It must be clear to anyone familiar with the so-called hemolytopoietic equilibrium, that the problem is only a little more than half solved when the cell production end of the mechanism alone has been considered. Optimum peripheral distribution and minimal destruction of the cells once delivered to the circulation are fully as important for the maintenance of health. The spleen acts physiologically as the chief sequestration and reservoir organ for the blood cells, and its normal content of phagocytic clasmatocytes is greater proportionately, and in the aggregate, than any other organ in the human body. This furnishes the back-

ground for the well known splenic function of senile cell disposition, but at the same time sets the stage for a series of disease entities dependent upon the pathologic overdevelopment of one or more of these normal "graveyard" functions. In congenital hemolytic icterus, if the spectacular clinical recovery which follows successful splenectomy,[29] is correctly interpreted, it is this surgical removal of an organ with an inherited tendency to excessive hemolysis, rather than any direct attack on bone marrow deficiency or inadequacy, which restores the cellular equilibrium. The bone marrow is hyperplastic for red cells at the late erythroblast and normoblast levels of maturation during active anemic episodes with jaundice, and in the absence of all splenic tissue, the marrow has been proved to be entirely competent to maintain a normal cellular balance, adequate for health and for resisting successfully infection, despite red cell size or fragility abnormalities.

In certain instances of thrombocytopenic purpura where the marrow can be shown to contain the usual, or excessive numbers of qualitatively normal megakaryocytes, the successful removal of the spleen restores promptly and permanently, an adequate supply of functionally normal platelets to the circulation.[40]

Finally, in selected patients showing a profound granulopenia of otherwise undetermined etiology, with or without hepatic cirrhosis, but practically always with a marked splenomegaly, a more or less specific phagocytosis of granulocytes by excessive numbers of clasmatocytes can be demonstrated, both histologically in the excised splenic tissue, and clinically through the subsequent restoration of a normal peripheral white cell count and differential, the bone marrow having been found to be hyperplastic for myeloid cells in each patient throughout the preoperative leukopenic episode.[41]

Thus, when any deficit occurs in the essential blood elements in the peripheral circulation, and bone marrow studies reveal an hyperplasia of qualitatively normal precursors, the organ of origin is automatically cleared in the great majority of instances, and some peripheral etiologic factor or factors must be sought. The more extensive our knowledge of the specificity of functions and sensitivity of response of the essential cells of blood and connective tissues, the more effective should become the scientific control of the great variety of noxious states most of which influence profoundly and are thereby in turn significantly affected by the reactions of the cells we have been considering. The problems of

functional differentiation and morphologic identification are no longer of academic interest only. The accumulated information now available, at its best is life saving and specifically curative when intelligently and discriminatingly applied; at second best, it places clinical prognosis on a sounder scientific basis and points the way to the next steps wherein may lie the more complete answers to the many questions and problems still challenging solution.

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